

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently Amended) A conjugate comprising a dye labeled nucleobase of the form:
 - (1) B-L-D, wherein B is a nucleobase, L is an anionic linker, and D comprises at least one fluorescent dye that comprises a xanthene, a rhodamine or a fluorescein, or
(2) B-L1-D1-L2-D2, wherein B is a nucleobase, L1 and L2 are linkers such that at least one of L1 and L2 is an anionic linker, and D1 and D2 are members of a fluorescent donor/acceptor pair, such that one of D1 and D2 is a donor dye capable of absorbing light at a first wavelength and emitting energy in response thereto, and the other of D1 and D2 is an acceptor dye capable of absorbing energy emitted by the donor dye and fluorescing at a second wavelength in response thereto, and at least one of D1 and D2 comprises a xanthene, a rhodamine or a fluorescein,
wherein L or at least one of L1 and L2 comprises at least one anionic phosphate or anionic phosphonate.
2. (Original) The conjugate of claim 1 wherein the dye-labeled nucleobase is of the form B-L-D.
3. (Original) The conjugate of claim 2 wherein L comprises a sulfonic acid moiety.
4. (Original) The conjugate of claim 2 wherein L comprises a sulfonated benzene moiety.
5. (Original) The conjugate of claim 2 wherein L comprises an anionic phosphate moiety.
6. (Original) The conjugate of claim 5 wherein the anionic phosphate moiety is a phosphate diester moiety, and the phosphorus atom of the phosphate diester moiety is located in L within a chain of linker atoms that connect B to D.

7. (Original) The conjugate of claim 2 wherein L comprises an anionic phosphonate moiety.
8. (Currently Amended) The conjugate of claim 7 wherein the anionic phosphonate moiety is a phosphonate monoester moiety, and the phosphorus atom of the phosphonate monoester moiety is located in L within a chain of linker atoms that connect B to D.
9. (Original) The conjugate of claim 2 wherein L comprises a carboxylic acid moiety.
10. (Original) The conjugate of claim 9 wherein the carboxylic acid moiety is a carboxyl benzene moiety.
11. (Original) The conjugate of claim 2 wherein L comprises 4 to 20 chain atoms.
12. (Cancel)
13. (Currently Amended) The conjugate of claim 42 2 wherein D comprises at least one fluorescein or rhodamine.
14. (Original) The conjugate of claim 1 wherein B comprises adenine, 7-deazaadenine, 7-deaza-8-azaadenine, cytosine, guanine, 7-deazaguanine, 7-deaza-8-azaguanine, thymine, uracil, or inosine.
15. (Original) The conjugate of claim 1 wherein the labeled nucleobase is of the form B-L1-D1-L2-D2.
16. (Cancel)
17. (Currently Amended) The conjugate of claim 46 15 wherein L1 comprises a sulfonic acid moiety.

18. (Currently Amended) The conjugate of claim 16 15 wherein L1 comprises a sulfonated benzene moiety.

19. (Currently Amended) The conjugate of claim 16 15 wherein L1 comprises an anionic phosphate moiety.

20. (Original) The conjugate of claim 19 wherein the anionic phosphate moiety is a phosphate diester moiety, and the phosphorus atom of the phosphate diester moiety is located in L1 within a chain of linker atoms that connect B to D1.

21. (Currently Amended) The conjugate of claim 16 15 wherein L1 comprises an anionic phosphonate moiety.

22. (Currently Amended) The conjugate of claim 21 wherein the anionic phosphonate moiety is a phosphonate monoester moiety, and the phosphorus atom of the phosphonate monoester moiety is located in L1 within a chain of linker atoms that connect B to D1.

23. (Currently Amended) The conjugate of claim 16 15 wherein L1 comprises a carboxylic acid moiety.

24. (Original) The conjugate of claim 23 wherein the carboxylic acid moiety is a carboxy benzene moiety.

25. (Original) The conjugate of claim 15 wherein L1 comprises 4 to 20 linker chain atoms.

26. (Cancel)

27. (Original) The conjugate of claim 15 wherein L2 is not an anionic linker.

28. (Original) The conjugate of claim 27 wherein D1-L2-D2 comprises structure (a), (b) or (c) below:

- (a) -D1-R₂₁Z₁C(O)R₂₂R₂₈-D2-
- (b) -D1-R₂₈R₂₂C(O)Z₁R₂₁-D2-
- (c) -D1-R₂₈R₂₂R₂₈-D2-

wherein:

R₂₁ is C₁-C₅ alkyldiyl,

Z₁ is NH, S, or O,

R₂₂ is ethenediyl, ethynediyl, 1,3-dienediyl, diynediyl, 1,3-diynediyl, a 5- or 6-membered ring having at least one unsaturated bond or a fused ring structure, and

R₂₈ is a bond or spacer group.

29. (Original) The conjugate of claim 28, wherein D1 is a donor dye and D2 is an acceptor dye.

30. (Original) The conjugate of claim 28 wherein R₂₂ is a five or six membered ring selected from the group consisting of cyclopentene, cyclohexene, cyclopentadiene, cyclohexadiene, furan, thifuran, pyrrole, isopyrrole, isoazole, pyrazole, isoimidazole, pyran, pyrone, benzene, pyridine, pyridazine, pyrimidine, pyrazine oxazine, indene, benzofuran, thionaphthene, indole and naphthalene.

31. (Original) The conjugate of claim 28 wherein R₂₈ is of the form R₂₉Z₂C(O), wherein R₂₉ is C₁-C₅ alkyldiyl, and Z₂ is NH, S, or O.

32. (Original) The conjugate of claim 28 wherein R₂₁ is CH₂, Z₁ is NH, R₂₂ is phena-1,4-diyl, and R₂₉ is CH₂, and Z₂ is NH.

33. (Original) The conjugate of claim 27 wherein L2 comprises up to 20 linker chain atoms.

34. (Original) The conjugate of claim 27 wherein D1 is a donor dye and D2 is an acceptor dye.

35. (Cancel)

36. (Currently Amended) The conjugate of claim 35 15 wherein L2 comprises a sulfonic acid moiety.

37. (Currently Amended) The conjugate of claim 35 15 wherein L2 comprises a sulfonated benzene moiety.

38. (Currently Amended) The conjugate of claim 35 15 wherein L2 comprises an anionic phosphate moiety.

39. (Original) The conjugate of claim 38 wherein the anionic phosphate moiety is a phosphate diester moiety, and the phosphorus atom of the phosphate diester moiety is located in L2 within a chain of linker atoms that connect D1 to D2.

40. (Currently Amended) The conjugate of claim 35 15 wherein L2 comprises an anionic phosphonate moiety.

41. (Currently Amended) The conjugate of claim 40 wherein the anionic phosphonate moiety is a phosphonate monoester moiety, and the phosphorus atom of the phosphonate monoester moiety is located in L2 within a chain of linker atoms that connect D1 to D2.

42. (Currently Amended) The conjugate of claim 35 15 wherein L2 comprises a carboxylic acid moiety.

43. (Original) The conjugate of claim 42 wherein the carboxylic acid moiety is a carboxy benzene moiety.

44. (Currently Amended) The conjugate of claim 35 15 wherein L2 comprises up to 20 linker chain atoms.

45. (Currently Amended) The conjugate of claim 35 15 wherein D1 is a donor dye and D2 is an acceptor dye.

46. (Original) The conjugate of claim 15 wherein L2 is an anionic linker and L1 is not an anionic linker.

47. (Original) The conjugate of claim 46 wherein L2 comprises a sulfonic acid moiety.

48. (Original) The conjugate of claim 46 wherein L2 comprises a sulfonated benzene moiety.

49. (Original) The conjugate of claim 46 wherein L2 comprises an anionic phosphate moiety.

50. (Original) The conjugate of claim 49 wherein the anionic phosphate moiety is a phosphate diester moiety, and the phosphorus atom of the phosphate diester moiety is located in L2 within a chain of linker atoms that connect D1 to D2.

51. (Original) The conjugate of claim 46 wherein L2 comprises an anionic phosphonate moiety.

52. (Currently Amended) The conjugate of claim 51 wherein the anionic phosphonate moiety is a phosphonate monoester moiety, and the phosphorus atom of the phosphonate monoester moiety is located in L2 within a chain of linker atoms that connect D1 to D2.

53. (Original) The conjugate of claim 46 wherein L2 comprises a carboxylic acid moiety.

54. (Original) The conjugate of claim 53 wherein the carboxylic acid moiety is a carboxy benzene moiety.

55. (Original) The conjugate of claim 46 wherein L2 comprises up to 20 linker chain atoms.

56. (Cancel)

57. (Original) The conjugate of claim 46 wherein D1 is a donor dye and D2 is an acceptor dye.

58. (Original) The conjugate of claim 46 wherein L1 comprises one of the following moieties:

-CC≡CH₂NH-, -C≡CCH₂NHC(O)(CH₂)₅NH-, -C=CC(O)NH(CH₂)₅NH-,
-C≡CCH₂OCH₂CH₂NH-, -C≡CCH₂OCH₂CH₂OCH₂CH₂NH-, -C≡C-CH₂OCH₂CH₂-NH-,
and -C≡C(p-C₄H₆)OCH₂CH₂NH-.

59.-81. (Cancel)

82. (Currently Amended) A method of sequencing a target polynucleotide sequence, the method comprising

(a) forming four classes of polynucleotides which are complementary to a target polynucleotide sequence, by template-dependent primer extension, wherein the polynucleotides in each class terminate with a different terminator subunit type that contains a distinct conjugate ~~of claim 1 comprising a dye labeled nucleobase of the form:~~

(1) B-L-D, wherein B is a nucleobase, L is an anionic linker, and D comprises at least one fluorescent dye that comprises a xanthene, a rhodamine or a fluorescein, or

(2) B-L1-D1-L2-D2, wherein B is a nucleobase, L1 and L2 are linkers such that at least one of L1 and L2 is an anionic linker, and D1 and D2 are members of a fluorescent donor/acceptor pair, such that one of D1 and D2 is a donor dye capable of absorbing light at a first wavelength and emitting energy in response thereto, and the other of D1 and D2 is an acceptor dye capable of absorbing energy emitted by the donor dye and fluorescing at a second wavelength in response thereto, and at least one of D1 and D2 comprises a xanthene, a rhodamine or a fluorescein,

wherein L or at least one of L1 and L2 comprises at least one anionic phosphate or anionic phosphonate to identify the polynucleotides in that class, and

(b) separating the polynucleotides of the four classes on the basis of size to obtain a mobility pattern, and determining the sequence of the target polynucleotide sequence from the mobility pattern.

83. (Original) The method of claim 82 wherein the terminator subunits are nonextendable.

84. (Original) The method of claim 82 wherein the terminator subunits contain a 3'-hydroxyl group.

85. (Cancel)

86. (New) The conjugate of claim 5 wherein the anionic phosphate moiety is a non-bridging phosphate moiety.

87. (New) The conjugate of claim 86 wherein the non-bridging phosphate moiety has a net charge of -1 or -2.

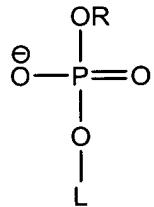
88. (New) The conjugate of claim 87 wherein the non-bridging phosphate moiety has a net charge of -1.

89. (New) The conjugate of claim 87 wherein the non-bridging phosphate moiety has a net charge of -2.

90. (New) The conjugate of claim 86 wherein the non-bridging phosphate moiety is a phosphate monoester or a phosphate diester.

91. (New) The conjugate of claim 96 wherein the non-bridging phosphate moiety is a phosphate monoester.

92. (New) The conjugate of claim 86 wherein the non-bridging phosphate moiety is a phosphate diester of the formula:



wherein R is a masking group selected from alkyl, alkenyl, alkynyl, aryl or alkaryl.

93. (New) The conjugate of claim 7 wherein the anionic phosphonate moiety is a non-bridging phosphonate moiety.

94. (New) The conjugate of claim 93 wherein the non-bridging phosphonate moiety has a net charge of -1 or -2.

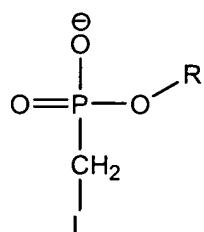
95. (New) The conjugate of claim 94 wherein the non-bridging phosphonate moiety has a net charge of -1.

96. (New) The conjugate of claim 94 wherein the non-bridging phosphonate moiety has a net charge of -2.

97. (New) The conjugate of claim 93 wherein the non-bridging phosphonate moiety is a phosphonic acid or a phosphonate monoester.

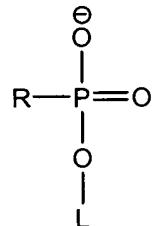
98. (New) The conjugate of claim 93 wherein the non-bridging phosphonate moiety is a phosphonic acid.

99. (New) The conjugate of claim 93 wherein the non-bridging phosphonate moiety is a phosphonate monoester of the formula:



wherein R is a masking group selected from alkyl, alkenyl, alkynyl, aryl or alkaryl.

100. (New) The conjugate of claim 93 wherein the non-bridging phosphonate moiety is a phosphonate monoester of the formula:



wherein R is a masking group selected from alkyl, alkenyl, alkynyl, aryl or alkaryl.

101. (New) The conjugate of claim 13 wherein D comprises at least one rhodamine dye.

102. (New) The conjugate of claim 13 wherein D comprises at least one fluorescein dye.

103. (New) The conjugate of claim 19 wherein the anionic phosphate moiety is a non-bridging phosphate moiety.

104. (New) The conjugate of claim 103 wherein the non-bridging phosphate moiety has a net charge of -1 or -2.

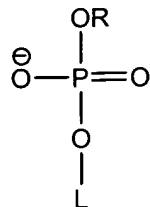
105. (New) The conjugate of claim 104 wherein the non-bridging phosphate moiety has a net charge of -1.

106. (New) The conjugate of claim 104 wherein the non-bridging phosphate moiety has a net charge of -2.

107. (New) The conjugate of claim 103 wherein the non-bridging phosphate moiety is a phosphate monoester or a phosphate diester.

108. (New) The conjugate of claim 103 wherein the non-bridging phosphate moiety is a phosphate monoester.

109. (New) The conjugate of claim 103 wherein the non-bridging phosphate moiety is a phosphate diester of the formula:



wherein R is a masking group selected from alkyl, alkenyl, alkynyl, aryl or alkaryl.

110. (New) The conjugate of claim 21 wherein the anionic phosphonate moiety is a non-bridging phosphonate moiety.

111. (New) The conjugate of claim 110 wherein the non-bridging phosphonate moiety has a net charge of -1 or -2.

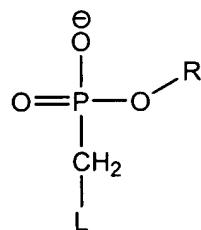
112. (New) The conjugate of claim 111 wherein the non-bridging phosphonate moiety has a net charge of -1.

113. (New) The conjugate of claim 111 wherein the non-bridging phosphonate moiety has a net charge of -2.

114. (New) The conjugate of claim 110 wherein the non-bridging phosphonate moiety is a phosphonic acid or a phosphonate monoester.

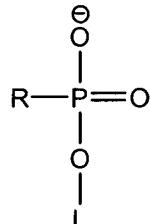
115. (New) The conjugate of claim 110 wherein the non-bridging phosphonate moiety is a phosphonic acid.

116. (New) The conjugate of claim 110 wherein the non-bridging phosphonate moiety is a phosphonate monoester of the formula:



wherein R is a masking group selected from alkyl, alkenyl, alkynyl, aryl or alkaryl.

117. (New) The conjugate of claim 110 wherein the non-bridging phosphonate moiety is a phosphonate monoester of the formula:



wherein R is a masking group selected from alkyl, alkenyl, alkynyl, aryl or alkaryl.

118. (New) The conjugate of claim 38 wherein the anionic phosphate moiety is a non-bridging phosphate moiety.

119. (New) The conjugate of claim 118 wherein the non-bridging phosphate moiety has a net charge of -1 or -2.

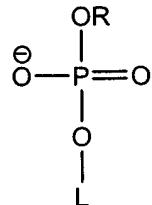
120. (New) The conjugate of claim 119 wherein the non-bridging phosphate moiety has a net charge of -1.

121. (New) The conjugate of claim 119 wherein the non-bridging phosphate moiety has a net charge of -2.

122. (New) The conjugate of claim 118 wherein the non-bridging phosphate moiety is a phosphate monoester or a phosphate diester.

123. (New) The conjugate of claim 118 wherein the non-bridging phosphate moiety is a phosphate monoester.

124. (New) The conjugate of claim 118 wherein the non-bridging phosphate moiety is a phosphate diester of the formula:



wherein R is a masking group selected from alkyl, alkenyl, alkynyl, aryl or alkaryl.

125. (New) The conjugate of claim 40 wherein the anionic phosphonate moiety is a non-bridging phosphonate moiety.

126. (New) The conjugate of claim 125 wherein the non-bridging phosphonate moiety has a net charge of -1 or -2.

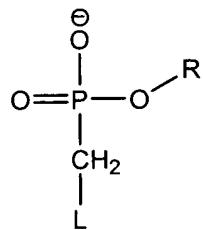
127. (New) The conjugate of claim 126 wherein the non-bridging phosphonate moiety has a net charge of -1.

128. (New) The conjugate of claim 126 wherein the non-bridging phosphonate moiety has a net charge of -2.

129. (New) The conjugate of claim 125 wherein the non-bridging phosphonate moiety is a phosphonic acid or a phosphonate monoester.

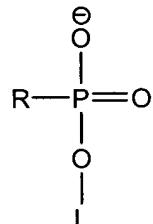
130. (New) The conjugate of claim 125 wherein the non-bridging phosphonate moiety is a phosphonic acid.

131. (New) The conjugate of claim 125 wherein the non-bridging phosphonate moiety is a phosphonate monoester of the formula:



wherein R is a masking group selected from alkyl, alkenyl, alkynyl, aryl or alkaryl.

132. (New) The conjugate of claim 125 wherein the non-bridging phosphonate moiety is a phosphonate monoester of the formula:



wherein R is a masking group selected from alkyl, alkenyl, alkynyl, aryl or alkaryl.

133. (New) The conjugate of claim 20 wherein L2 comprises a sulfonic acid moiety.

134. (New) The conjugate of claim 20 wherein L2 comprises a sulfonated benzene moiety.

135. (New) The conjugate of claim 22 wherein L2 is not an anionic linker.

136. (New) The method of claim 82 wherein the dye-labeled nucleobase is of the form B-L-D.

137. (New) The method of claim 136 wherein L comprises a sulfonic acid moiety.

138. (New) The method of claim 136 wherein L comprises a sulfonated benzene moiety.

139. (New) The method of claim 136 wherein L comprises an anionic phosphate moiety.

140. (New) The method of claim 139 wherein the anionic phosphate moiety is a phosphate diester moiety, and the phosphorus atom of the phosphate diester moiety is located in L within a chain of linker atoms that connect B to D.

141. (New) The method of claim 136 wherein L comprises an anionic phosphonate moiety.

142. (New) The method of claim 141 wherein the anionic phosphonate moiety is a phosphonate monoester moiety, and the phosphorus atom of the phosphonate monoester moiety is located in L within a chain of linker atoms that connect B to D.

143. (New) The method of claim 136 wherein L comprises a carboxylic acid moiety.

144. (New) The method of claim 143 wherein the carboxylic acid moiety is a carboxyl benzene moiety.

145. (New) The method of claim 136 wherein L comprises 4 to 20 chain atoms.

146. (New) The method of claim 136 wherein D comprises at least one fluorescein or rhodamine.

147. (New) The method of claim 82 wherein B comprises adenine, 7-deazaadenine, 7-deaza-8-azaadenine, cytosine, guanine, 7-deazaguanine, 7-deaza-8-azaguanine, thymine, uracil, or inosine.

148. (New) The method of claim 82 wherein the labeled nucleobase is of the form B-L1-D1-L2-D2.

149. (New) The method of claim 148 wherein L1 comprises a sulfonic acid moiety.

150. (New) The method of claim 148 wherein L1 comprises a sulfonated benzene moiety.

151. (New) The method of claim 148 wherein L1 comprises an anionic phosphate moiety.

152. (New) The method of claim 151 wherein the anionic phosphate moiety is a phosphate diester moiety, and the phosphorus atom of the phosphate diester moiety is located in L1 within a chain of linker atoms that connect B to D1.

153. (New) The method of claim 148 wherein L1 comprises an anionic phosphonate moiety.

154. (New) The method of claim 153 wherein the anionic phosphonate moiety is a phosphonate monoester moiety, and the phosphorus atom of the phosphonate monoester moiety is located in L1 within a chain of linker atoms that connect B to D1.

155. (New) The method of claim 148 wherein L1 comprises a carboxylic acid moiety.

156. (New) The method of claim 155 wherein the carboxylic acid moiety is a carboxy benzene moiety.

157. (New) The method of claim 148 wherein L1 comprises 4 to 20 linker chain atoms.

158. (New) The method of claim 148 wherein L2 is not an anionic linker.

159. (New) The method of claim 158 wherein L2 comprises up to 20 linker chain atoms.

160. (New) The method of claim 158 wherein D1 is a donor dye and D2 is an acceptor dye.

161. (New) The method of claim 148 wherein L2 comprises a sulfonic acid moiety.

162. (New) The method of claim 148 wherein L2 comprises a sulfonated benzene moiety.

163. (New) The method of claim 148 wherein L2 comprises an anionic phosphate moiety.

164. (New) The method of claim 163 wherein the anionic phosphate moiety is a phosphate diester moiety, and the phosphorus atom of the phosphate diester moiety is located in L2 within a chain of linker atoms that connect D1 to D2.

165. (New) The method of claim 148 wherein L2 comprises an anionic phosphonate moiety.

166. (New) The method of claim 165 wherein the anionic phosphonate moiety is a phosphonate monoester moiety, and the phosphorus atom of the phosphonate monoester moiety is located in L2 within a chain of linker atoms that connect D1 to D2.

167. (New) The method of claim 148 wherein L2 comprises a carboxylic acid moiety.

168. (New) The method of claim 167 wherein the carboxylic acid moiety is a carboxy benzene moiety.

169. (New) The method of claim 148 wherein L2 comprises up to 20 linker chain atoms.

170. (New) The method of claim 148 wherein D1 is a donor dye and D2 is an acceptor dye.

171. (New) The method of claim 148 wherein L2 is an anionic linker and L1 is not an anionic linker.

172. (New) The method of claim 171 wherein L2 comprises a sulfonic acid moiety.

173. (New) The method of claim 171 wherein L2 comprises a sulfonated benzene moiety.

174. (New) The method of claim 171 wherein L2 comprises an anionic phosphate moiety.

175. (New) The method of claim 174 wherein the anionic phosphate moiety is a phosphate diester moiety, and the phosphorus atom of the phosphate diester moiety is located in L2 within a chain of linker atoms that connect D1 to D2.

176. (New) The method of claim 171 wherein L2 comprises an anionic phosphonate moiety.

177. (New) The method of claim 176 wherein the anionic phosphonate moiety is a phosphonate monoester moiety, and the phosphorus atom of the phosphonate monoester moiety is located in L2 within a chain of linker atoms that connect D1 to D2.

178. (New) The method of claim 171 wherein L2 comprises a carboxylic acid moiety.

179. (New) The method of claim 178 wherein the carboxylic acid moiety is a carboxy benzene moiety.

180. (New) The method of claim 171 wherein L2 comprises up to 20 linker chain atoms.

181. (New) The method of claim 171 wherein D1 is a donor dye and D2 is an acceptor dye.